Can high risk fungicides be used in mixtures without selecting for fungicide resistance?

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Abstract

Fungicide mixtures produced by the agrochemical industry often contain low risk fungicides, to which fungal pathogens are fully sensitive together with high risk fungicides known to be prone to fungicide resistance. Can these mixtures provide adequate disease control while minimizing the risk for the development of resistance? We present a population dynamics model to address this question. We found that the fitness cost of resistance is a crucial parameter to determine the outcome of competition between the sensitive and resistant pathogen strains and to assess the usefulness of a mixture. If fitness costs are absent, then the use of the high risk fungicide in a mixture selects for resistance and the fungicide eventually becomes nonfunctional. If there is a cost of resistance, then an optimal ratio of fungicides in the mixture can be found, at which selection for resistance is expected to vanish and the level of disease control optimized.

1. Introduction

Fungal pathogens of plants pose a serious problem for sustainable food production [1]. In order to protect crops from fungal diseases, farmers routinely apply fungicide sprays. However, in many cases plant pathogenic fungi develop resistance to one or more fungicides or fungicide classes, which makes the treatment ineffective and may lead to severe crop losses and lower profit for farmers.

Fungicide resistance is a prime example of adaptation of a population to an environmental change, also known as evolutionary rescue [2, 3]. While global climate change is expected to result in a loss of biodiversity in natural ecosystems, evolutionary rescue is seen as a mechanism that may mitigate this loss. In the context of crop protection the point of view is quite the opposite: adaptation of pathogens of crop plants to the use of chemical disease control measures is seen as a major threat to food production. Therefore, any methods that diminish the adaptive ability of pathogens and decrease their diversity would be helpful to develop better crop protection strategies. This requires a detailed quantitative understanding of the dynamics of infection and the factors driving the emergence and development of fungicide resistance [4]. Despite the global importance and urgency of fungicide resistance, this problem has received relatively little theoretical consideration (see [5, 6, 7, 8, 9] and [4] for a comprehensive review).

In recent years, agrochemical companies have begun marketing mixtures that contain fungicides with a low risk of resistance with fungicides that have a high risk of resistance. In extreme cases the high risk fungicide is no longer effective against some common pathogens because resistance has become widespread. For example, a large proportion of the European population of the important wheat pathogen *Mycosphaerella graminicola* (recently renamed *Zymoseptoria tritici*) [10, 11] is resistant to strobilurin fungicides [12] but farmers continue to use the strobilurins in combination with other fungicides because strobilurins are thought to contribute additional physiological benefits or to protect the crop from other pathogens.

Can the application of mixtures of low risk and high risk fungicides bring any advantage in terms of crop protection while minimizing further selection for resistance? Under which conditions does this type of mixture add anything to the treatment benefit, compared to when a low risk fungicide is applied alone? We addressed these questions using a simple population dynamics model of host-pathogen interaction based on a system of ordinary differential equations. We found that the fitness cost associated with resistance mutations is a crucial parameter, which governs the outcome of the competition between the sensitive and resistant pathogen strains.

In the absence of a fitness cost, application of a high risk fungicide in a mixture inevitably and irreversibly selects for resistance, which makes its use in the mixture ineffective. On the other hand, if the resistance mechanism does confer a significant fitness cost to the pathogen, then application of the fungicide mixture may provide a stable advantage. In this case, an optimal ratio of the high risk fungicide in the mixture can be found below which the application of a high risk fungicide is beneficial, but at the same time does not select for resistance. The optimal ratio is determined by the cost of resistance, the degree of pharmacological interaction between the fungicides,

and depends on the degree of resistance for the case of partial resistance. Therefore, knowledge of the cost of resistance is necessary to determine whether the application of a given fungicide mixture will select for the resistant strain.

A single point mutation associated with fungicide resistance often makes the pathogen completely insensitive to a fungicide, as is the case for the G143A mutation giving resistance to strobilurin fungicides in many fungal pathogens [13, 14]. In many other cases the resistance is partial and has properties of a quantitative trait, for example, resistance of *Z. tritici* and other fungi to azole fungicides [15]. It was found that the resistance in *Z. tritici* results from accumulation of many mutations of the *CYP51* gene, which encodes for the sterol 14α-demethylase, an enzyme involved in sterol biosynthesis and the target protein of azoles [16, 17, 18]. In order to account for these features, we have considered varying degrees of resistance and fitness costs in our model.

Most of the previous modeling studies on the effect of fungicide mixtures on the development of the resistance were based on "density-independent" models, in which the pathogen population is assumed to grow exponentially over time and the effect of a finite host population is neglected (for example, [19, 20, 21]). However, the actual disease progress curves can usually be approximated by an exponential function only at the very beginning of an epidemic [22]. In a recent study [5], a more realistic model was considered, in which the finiteness of the host tissue susceptible to infection was taken into account. However, in contrast to our study, resistance was assumed to bear no fitness costs for the pathogen. It was found that in the absence of fitness costs the use of fungicide mixtures *delays* the development of resistance [5]. This conclusion is in agreement with our results. In the absence of fitness costs the application of the high risk fungicide gives a selective advantage to the resistant pathogen strain. But the rate at which the frequency of the resistant strain increases is proportional to the difference in fitness between the resistant and sensitive pathogen strains: the larger the difference, the faster is the selection. The sensitive pathogen strain is suppressed in the same way by the high risk and the low risk fungicide, therefore, its fitness is the same when the high risk fungicide is applied alone and when a mixture of the two fungicides is applied. In contrast, the fitness of the resistant strain is diminished to a greater extent by the mixture than by the high risk fungicide alone. Thus, the difference in fitness between the two strains is smaller and the selection for resistance is delayed when the mixture is applied (see Appendix A.4 in the Electronic Supplementary Material for more details). Here we focus on finding conditions under which the selection for the resistant pathogen strain is *prevented* rather than delayed by using fungicide mixtures.

The problem of combining chemical biocides in order to delay or prevent the development of resistance also appears in other contexts, such as resistance of agricultural weeds to herbicides [23], insect pests to insecticides [24], and human pathogens and diseases including HIV [25], bacterial infections [26], malaria [27] and cancer [28]. Accordingly, lessons from fungicide combinations may well apply to the problem of biocide resistance in many other contexts.

2. Model and assumptions

In order to investigate the effect of fungicide mixtures on selection for fungicide resistance, we use a deterministic mathematical model of susceptible-infected dynamics (see Fig. 1)

$$\frac{dH}{dt} = r(K - H - I_\mathrm{s} - I_\mathrm{r}) - b\left[I_\mathrm{s} + (1-\rho_\mathrm{r})I_\mathrm{r}\right]H, \tag{1}$$

$$\frac{dI_\mathrm{s}}{dt} = bHI_\mathrm{s} - \left[1 + \varepsilon_\mathrm{s}(C, r_\mathrm{B})\right]\mu I_\mathrm{s}, \tag{2}$$

$$\frac{dI_\mathrm{r}}{dt} = b(1-\rho_\mathrm{r})HI_\mathrm{r} - \left[1 + \varepsilon_\mathrm{r}(C, r_\mathrm{B})\right]\mu I_\mathrm{r}. \tag{3}$$

The model has three compartments: susceptible hosts H, hosts infected by a sensitive pathogen strain I_s, hosts infected by a resistant pathogen strain I_r; and is similar to the models described by [4, 6]. The subscript "s" stands for the sensitive strain and the subscript "r" stands for the resistant strain. The quantities H, I_s and I_r, represent the total amount of the corresponding host tissue within one field, which could be leaves, stems or grain tissue, depending on the specific host-pathogen interaction. Susceptible hosts H grow with the rate r. Their growth is limited by the "carrying capacity" K, which may imply limited space or nutrients. Furthermore, susceptible hosts may be infected by the sensitive pathogen strain and transformed into infected hosts in the compartment I_s with the transmission rate b. Susceptible hosts may also be infected by the resistant pathogen strain and transformed into infected hosts in the compartment I_r. In this case, the resistant strain suffers a fitness cost ρ_r which affects its transmission rate such that it becomes equal to $b(1-\rho_\mathrm{r})$. The corresponding terms in Eqs. (1)-(3) are proportional to the amount of the available susceptible tissue H and to the amount of the infected tissue I_s or I_r. Infected host tissue loses its infectivity at a rate μ due to pathogen death.

Since our description is deterministic we do not take into account the emergence of new resistance mutations but assume that the resistant pathogen strain (I_r) is already present in the population. Therefore, when "selection for resistance" is discussed below, we refer to the process of winning the competition by this given resistant strain due to its higher fitness with respect to the sensitive strain in the presence of fungicide treatment. Emergence of new resistance mutations is a different problem, which goes beyond the scope of our study and requires stochastic simulation methods. We do not consider the possibility of double resistance in the model, but by preventing selection for single resistance as described here, one would also diminish the probability of the emergence of double resistance for both sexually and asexually reproducing pathogens (see Appendix A.7).

We consider two fungicides A and B. The fungicide A is the high risk fungicide, to which the resistant pathogen strain exhibits a variable degree of resistance. However, the sensitive strain is fully sensitive to fungicide A. The fungicide B is the low risk fungicide, i. e. both pathogen strains are fully sensitive to it. We compare the effect of

the fungicide A applied alone, fungicide B applied alone and the effect of their mixture with an arbitrary proportion. We assume that the fungicides will increase the pathogen death rate [see the expression in square brackets in Eq. (2), Eq. (3)]. Whether only one or a mixture of two fungicides is applied, the general form of the fungicide action is described by

$$\varepsilon(C) = k_{\mathrm{k}} \frac{C}{C + C_{50}} \tag{4}$$

This function grows with the fungicide concentration C and saturates at a value k_{k}, the maximum killing rate. The parameter C_{50} is the fungicide concentration at which half of the maximum effect is achieved; it determines how fast the function $\varepsilon(C)$ grows. We assume that the action of the fungicide A when applied alone is described by Eq. (4): $\varepsilon(C_{\mathrm{A}}) = k_{\mathrm{kA}} C_{\mathrm{A}}/(C_{\mathrm{A}} + C_{50\mathrm{A}})$ and the action of the fungicide B when applied alone is also given by Eq. (4): $\varepsilon(C_{\mathrm{B}}) = k_{\mathrm{kB}} C_{\mathrm{B}}/(C_{\mathrm{B}} + C_{50\mathrm{B}})$. For simplicity we assume that $k_{\mathrm{kA}} = k_{\mathrm{kB}} = k_{\mathrm{k}}$ and $C_{50\mathrm{A}} = C_{50\mathrm{B}} = C_{50}$. If the parameters $C_{50\mathrm{A}}$ and $C_{50\mathrm{B}}$ are different, the concentration axis can be rescaled so that they become the same. A more general case, where the maximum killing rates k_{kA} and k_{kB} are different can also be considered, but leads to slightly more complicated expressions. The function $\varepsilon(C)$ in Eq. (4) is a special case of the sigmoid Emax model [29] with the Hill coefficient equal to one, which is often used in the antibiotic [30] and fungicide resistance literature [6].

When a mixture of two fungicides is applied, it increases the death rate of the sensitive pathogen according to

$$\varepsilon_{\mathrm{s}}(C, r_{\mathrm{B}}) = k_{\mathrm{k}} \frac{C}{C + C_{50}/\gamma_{\mathrm{s}}}, \tag{5}$$

and the death rate of the resistant pathogen is increased by

$$\varepsilon_{\mathrm{r}}(C, r_{\mathrm{B}}) = k_{\mathrm{k}} \frac{C}{C + C_{50}/\gamma_{\mathrm{r}}}. \tag{6}$$

Here $C = C_{\mathrm{A}} + C_{\mathrm{B}}$, where C_{A} is the concentration of the fungicide A and C_{B} is the concentration of the fungicide B, $r_{\mathrm{B}} = C_{\mathrm{B}}/C$ is the proportion of the fungicide B in the mixture and

$$\gamma_{\mathrm{s}} = 1 + u\sqrt{r_{\mathrm{B}}(1 - r_{\mathrm{B}})}, \tag{7}$$

$$\gamma_{\mathrm{r}} = \alpha(1 - r_{\mathrm{B}}) + r_{\mathrm{B}} + u\sqrt{\alpha r_{\mathrm{B}}(1 - r_{\mathrm{B}})} \tag{8}$$

are the parameters which modify C_{50} due to pharmacological interaction and partial resistance. The Eqs. (5), (6) are obtained from more general Eqs. (A.4), (A.5) by changing the variables from C_{A}, C_{B} to C, r_{B} and assuming that the sensitive pathogen strain is fully sensitive to both fungicides and the resistant strain can have a varying degree of sensitivity α to the fungicide A, but is fully sensitive to the fungicide B. The degree of sensitivity α enters as the coefficient before $(1 - r_{\mathrm{B}})$ in Eq. (8) and lies between zero and one. At $\alpha = 0$ the pathogen is fully resistant to fungicide A, and at $\alpha = 1$ the pathogen is fully sensitive to it.

The degree of pharmacological interaction is characterized by the parameter u. At $u = 0$ the fungicides do not interact, $u > 0$ represents synergy and $u < 0$ is the case of antagonism. (See Appendix A.1 for the discussion of pharmacological interaction.) Note that the term responsible for the fungicide interaction in Eqs. (7), (8) is proportional to $\sqrt{r_\mathrm{B}(1 - r_\mathrm{B})}$, which is zero when only one of the fungicides is applied, i. e. at $r_\mathrm{B} = 0$ or $r_\mathrm{B} = 1$, and reaches a maximum when the two fungicides are mixed in equal proportions, i. e. $r_\mathrm{B} = 1/2$.

We consider one growing season and assume the host-pathogen equilibrium to be reached during the season. That corresponds to the time-dependent solution of Eqs. (1)-(3) reaching its stable steady state. When saturation of the empirical disease progress curve occurs at a value below 100 %, this is a good indication that the equilibrium is reached during one season. Whether this is the case depends on both the host-pathogen combination and the environmental conditions. While in some cases the disease progress curve saturates (for example, [31, 22]), in other cases it does not (for example, [32, 22]).

We assume that the fungicide concentration is constant over time and neglect the spatial dependencies of the variables H, I_s and I_r and all other parameters. The latent phase of infection, which could be considerable for some pathogens (for example, *Z. tritici* has a latent period of about 12 days), is also neglected. Since we neglect mutation, migration and spatial heterogeneity, the two pathogen strains cannot co-exist in the long-term.

Such competitive exclusion is observed in the field in some cases, but in other cases resistant and sensitive pathogen strains are found to coexist. For example, a gradual increase in the frequency of fungicide-resistant pathogen strains up to almost 100 % was observed in field experiments in plots treated with fungicides, but a stable co-existence of resistant and pathogen strains developed in untreated plots [33]. In a field monitoring study [34] a fungicide-resistant pathogen strain emerged under fungicide treatment and gradually dominated the pathogen population over the course of five years. A modeling study [7] has shown that spatial inhomogeneity in the form of imperfect fungicide coverage makes the stable co-existence of resistant and sensitive pathogen strains possible if they have similar values of the basic reproductive number.

The basic reproductive number (denoted as R_0) is often used in epidemiology as a measure of transmission fitness of infectious pathogens [35]. It is defined as the expected number of secondary infections resulting from a single infected individual introduced into a susceptible population. At $R_0 > 1$ the infection can spread over the population, while at $R_0 < 1$ it cannot and the epidemic dies out.

The equilibrium stability analysis of the model Eqs. (1)-(3) shows that the relationship between the basic reproductive number of the sensitive strain

$$R_{0\mathrm{s}} = \frac{bK}{[1 + \varepsilon_\mathrm{s}(C, r_\mathrm{B})]\,\mu} \tag{9}$$

and the basic reproductive number of the resistant strain

$$R_{0\mathrm{r}} = \frac{b(1-\rho_{\mathrm{r}})K}{[1+\varepsilon_{\mathrm{r}}(C, r_{\mathrm{B}})]\,\mu} \tag{10}$$

determines the long-term outcome of the epidemic. The sensitive strain wins the competition and dominates the pathogen population if $R_{0\mathrm{s}} > 1$, such that it can survive in the absence of the resistant strain, and $R_{0\mathrm{s}} > R_{0\mathrm{r}}$, such that is has a selective advantage over the resistant strain. The second inequality is equivalent to

$$\frac{C}{C + C_{50}/\gamma_{\mathrm{s}}} < \left(\rho_{\mathrm{r}}/k_{\mathrm{k}} + \frac{C}{C + C_{50}/\gamma_{\mathrm{r}}}\right)/(1-\rho_{\mathrm{r}}). \tag{11}$$

Similarly, the resistant strain wins the competition and dominates the population if $R_{0\mathrm{r}} > 1$ and $R_{0\mathrm{r}} > R_{0\mathrm{s}}$.

We assumed here that resistance cost decreases transmission rate b of the resistant pathogen strain and the fungicide increases the pathogen death rate μ. We performed the same equilibrium analysis when the effect of the resistance cost and the fungicide enter the model in other ways and obtained qualitatively the same results (see Appendix A.5)

The simplicity of the model allows us to obtain all the results analytically. We determined explicit mathematical relationships between the quantities of interest, which enabled us to study the effects over the whole range of parameters.

3. Results

Understanding the factors determining the outcome of the competition between sensitive and resistant pathogen strains is important for developing an optimal treatment regime. Hence, we first investigate the parameter ranges over which resistant or sensitive strains dominate the pathogen population for the case of fungicides A and B applied individually and for the mixture of the two fungicides (Sec. 3.1). Then, we consider the optimal proportion of fungicides to include in a mixture in Sec. 3.2 and the benefit of fungicide treatment in Sec. 3.3. Finally, we take into account possible pharmacological interactions between fungicides and consider the effect of partial resistance (Sec. 3.4, 3.5).

3.1. Selection for resistance

The ranges of fungicide concentration and cost of resistance at which the sensitive (white) or resistant (dark red) pathogen strain is favored by selection are shown in Fig. 2. If a low risk fungicide is applied alone, the sensitive strain has a selective advantage across the whole parameter range in Fig. 2(a). When only a high risk fungicide is applied [Fig. 2(b)] the resistance dominates if the fitness cost is low $\rho_{\mathrm{r}} < k_{\mathrm{k}}/(k_{\mathrm{k}}+1)$ and at a fungicide concentration higher than a threshold value which increases with the fitness cost [see Eq. (A.22)]. If the fitness cost exceeds $k_{\mathrm{k}}/(k_{\mathrm{k}}+1)$ [dotted vertical line in Fig. 2(b)], then the sensitive strain dominates at any fungicide concentration.

The Fig. 2(c) shows the outcome when the two fungicides are mixed at equal concentrations: here the picture changes considerably. Now the borderline between the area where the sensitive strain dominates at any fungicide concentration and the area where resistance may dominate, is shifted to smaller values. Moreover, to the left of the borderline, an area where the sensitive strain dominates appears at concentrations above a threshold value.

Importantly, without a fitness cost ($\rho_\mathrm{r} = 0$) the resistant strain becomes favored by selection and will eventually dominate the population whenever the high risk fungicide is applied, alone or in combination with the low risk fungicide [Fig. 2(b,c)].

Interestingly, the value of the borderline [vertical dotted line in Fig. 2(c)] depends on the ratio of the fungicides in the mixture. This allows one to determine an optimal proportion of fungicides to use in the mixture (see Sec. 3.2).

3.2. Optimal proportion of fungicides in a mixture

It is highly desirable to keep existing fungicides effective for as long as possible. Therefore, an optimal mixture contains the largest proportion of the high risk fungicide, at which (i) the resistant pathogen strain is not selected and (ii) an adequate level of disease control is achieved. In order to fulfill both of these objectives, it is necessary for the fitness cost of resistance to be larger than a threshold value $\rho_\mathrm{r} > \rho_\mathrm{rb}$ [see Eq. (A.13) in Appendix]. This means that selection is operating to the right of the vertical dashed line in Fig. 2(c), where the sensitive strain is favored and the resistant strain is eliminated at any fungicide concentration.

The threshold value ρ_rb depends on the proportion of fungicides in the mixture. Adding more of the low risk fungicide, while keeping the total concentration C constant, shifts the threshold to the left. This diminishes the range of the values for fitness cost at which the resistant strain is competitively superior. On the other hand, adding less of the low risk fungicide, while again keeping C constant, shifts the border to the right, increasing the parameter range over which the resistant strain is favored.

Therefore, at a given fitness cost ρ_r, one can adjust the fungicide ratio r_B in such a way that $\rho_\mathrm{r} > \rho_\mathrm{rb}$. This is shown in Fig. 3: the curve shows the critical proportion of the low risk fungicide r_Bc, above which the treatment with a mixture is effective in the long run, meaning no selection for resistance occurs at any total fungicide concentration C. One can see from Fig. 3 that if the resistance cost is zero ($\rho_\mathrm{r} = 0$), then for the treatment to be effective the fungicide A should not be added at all, i. e. $r_\mathrm{B} = 1$. As the fitness cost grows, the value of r_Bc decreases, giving the possibility to use a larger proportion of the high risk fungicide without selecting for resistance.

So far we have shown how choosing an optimal proportion of fungicides in the mixture prevents selection for resistance. Now, we will consider how to achieve an adequate level of disease control. In order to do this, we need to quantify the benefit of fungicide treatment.

3.3. Treatment benefit

The yield of cereal crops is usually assumed to be proportional to the healthy green leaf area, which corresponds in our model to the amount of susceptible hosts $H(t)$. Accordingly, we quantify the benefit of the fungicide treatment as the ratio between the amount of susceptible hosts $H(t)$ when both the disease and treatment are present and its value $H_{\mathrm{nd}}(t)$ in the absence of disease: $B(t) = H(t)/H_{\mathrm{nd}}(t)$. Hence, $B(t) = 1$ corresponds to a perfect treatment, which eradicates the disease completely and the treatment benefit of zero corresponds to a situation where all susceptible hosts are infected by disease. This quantity characterizes the effectiveness of the chemical disease control.

The treatment benefit at equilibrium as a function of the fitness cost and the fungicide concentration is shown in Fig. 4. (The corresponding equations are given in Appendix A.3.) The range of fitness costs and fungicide concentrations is the same as in Fig. 2. The upper graph shows the outcome when a low risk fungicide is applied alone. Here, the treatment benefit does not depend on the cost of resistance, but increases monotonously with the fungicide concentration, since the sensitive strain is favored by selection here.

The dependency changes when a high risk fungicide is applied alone (middle graph): the benefit increases with the fungicide concentration and does not depend on the cost of resistance only in the parameter range where the sensitive strain is favored (to the right from the black solid curve). In the range where resistance dominates, the treatment benefit depends only on the cost of resistance and is independent of the fungicide concentration, as we assume resistant mutants are fully protected from the fungicide A. Hence, increasing its concentration does not affect the amount of disease.

In the case of a mixture of a high risk and a low risk fungicide, the parameter range in which the treatment benefit depends only on the fungicide concentration and is independent of the cost of resistance becomes larger. In the area where resistance is favored (to the left from the black solid curve) the treatment benefit increases with both the cost of resistance and the fungicide concentration, since the fungicide B in the mixture suppresses the resistant strain.

Both fungicides in the mixture contribute to the suppression of the disease only in the region of Fig. 4(c), where the sensitive strain dominates (to the right from the black solid curve). Moreover, to achieve the desired treatment benefit by adjusting the total fungicide concentration C without the danger of selecting for resistance, the fitness cost should exceed its threshold value $\rho_{\mathrm{r}} > \rho_{\mathrm{rb}}$ [to the right from the dashed vertical line in Fig. 4(c)]. As we have shown above, the threshold value of the resistance cost ρ_{rb} depends on the proportion of fungicides in a mixture. Thus by first choosing an optimal proportion of fungicides r_{B} as suggested in Sec. 3.2 and then choosing the total fungicide concentration such that the treatment benefit reaches a high enough value, one can avoid selection for resistance and at the same time reach the desired level of disease control.

3.4. The effect of pharmacological interaction between fungicides

Synergistic interactions between fungicides makes their combined effect greater than expected with purely additive interactions. The sensitive pathogen strain is suppressed more effectively by a synergistic mixture, while the resistant strain is not affected by the interaction (in case of full resistance $\alpha = 0$). This increases the range of fitness costs over which resistance has a selective advantage [the dashed vertical line in Fig. 2(c) shifts to the right]. Consequently, the critical proportion of the low risk fungicide in the mixture r_{Bc}, above which the resistant mutants are eliminated increases [dotted curve in Fig. 5(a)]. In contrast, an antagonistic mixture suppresses the sensitive strain less effectively than either fungicide used alone. Hence the range of fitness costs over which resistance dominates becomes smaller and the ratio r_{Bc} decreases [dashed curve in Fig. 5(a)]. This result is in agreement with studies on drug interactions in the context of antibiotic resistance, where antagonistic drug combinations were found to select against resistant bacterial strains [36].

3.5. The effect of partial fungicide resistance

Consider the situation when the resistant pathogen strain is not fully protected from the high risk fungicide, but exhibits a partial resistance ($0 < \alpha < 1$). In this case, the fungicide mixture is more effective in suppressing the resistant strain than in the case of full resistance ($\alpha = 0$) considered above. Therefore, one needs less of the low risk fungicide in the mixture to reach the conditions where resistance is eliminated by selection: the critical proportion of the low risk fungicide in the mixture decreases with the degree of sensitivity α in Fig. 5(b). Also, in Fig. 5(a) the dependency of the critical ratio of the fungicide B in the mixture for partial resistance (light grey curve) lies below the one at perfect resistance and reaches zero at a much smaller value. Thus, knowledge of the degree of resistance is crucial for determining an appropriate proportion of fungicides in the mixture.

4. Discussion and conclusions

The above results demonstrate that if fungicide resistance comes without a fitness cost, application of fungicides prone to resistance (high risk fungicides) in a mixture with fungicides still free from resistance (low risk fungicides) will select for resistance. Consequently, as soon as the equilibrium is reached the resistant strain will dominate the pathogen population and the sensitive strain will be eliminated. Because of this, the high risk fungicide will not affect the amount of disease and only the low risk fungicide component of the mixture will be acting against disease. Hence, the high risk fungicide becomes nonfunctional in the mixture and using the low risk fungicide alone would have the same effect at a lower cost.

We extensively searched the available literature on fitness costs in different fungal pathogens of crop plants. A few studies inferred significant fitness costs from field monitoring (see for example, [37] and references in [38]). But the findings in these studies

could also be due to other factors, including immigration of sensitive isolates, selection for other traits linked to resistance mutations or genetic drift [38]. Though relatively few carefully controlled experiments have been conducted, the majority indicate that fitness costs associated with fungicide resistance are either low (for example, [39, 40]) or absent (for example, [41, 42]). Moreover, even if fitness costs are measured in resistant mutants generated in the lab [39], they might not represent those selected in the field, because compensatory mutations improving pathogen fitness are likely to occur under field conditions [38]. Thus the use of fungicide mixtures that include high-risk or already failed fungicides will select for resistance for most plant pathogens and cannot usually be justified according to the results from our model and current knowledge on fitness costs. But in the rare cases where fitness costs are significant (for example [43]), high risk fungicides in a mixture with low risk fungicides are expected to contribute to the suppression of disease and not to select for resistance if used at a low enough proportion.

However, current knowledge of fitness costs is insufficient, since they may manifest in many different ways (for example, by impairing the ability of a pathogen to overseason [44]) and depend on environmental conditions. We hope this study will stimulate further experimental investigations to better characterize them and expect that significant costs will be found in some cases. In these cases, high risk fungicides can be used effectively for an extended period of time. An optimal proportion of the high risk fungicide in a mixture with the low risk fungicide can be determined that contains as much as possible of the high risk fungicide, but still does not select for resistance while providing adequate disease control (see Box 1). If a mixture with the optimum proportion is applied, then the rise of the resistant strain is prevented for an unlimited time. Under the scenario where the resistant pathogen strain is fully protected from the high risk fungicide, the fungicides do not interact and the fitness cost of resistance decreases transmission rate b by 5 percent, then up to 20 percent of the high risk fungicide can used in the mixture without selecting for resistance (Fig. 3).

While it was previously discussed [45] that alternation of high risk and low risk fungicides might be a useful tactic for disease control in the presence of a fitness cost, we have shown that a mixture of these fungicides in an appropriate proportion can provide adequate disease control without selecting for resistance. Mixtures offer an advantage compared to alternation because there is no need to delay the application of the high risk fungicide and the resistant strain does not rise to high frequencies, which lowers the risk of its further spread (see Appendix A.6).

This analysis applies only for a known resistant strain or mutation. If a new resistance mutation emerges with a different fitness cost, then it should also be taken into account when determining the optimal proportion of fungicides in the mixture. Alternatively, if a compensatory mutation emerges which strongly diminishes the fitness cost of resistance, then the resistant strain carrying the compensatory mutation may rise in frequency and eventually outcompete the sensitive strain.

Our model considered only one pathogen that has a resistant strain, but there can be several different pathogens with resistant strains infecting a given host population. To minimize costs farmers prefer to use the same fungicide mixture to control as many pathogens as possible. Our approach could be generalized for this case: a proportion of

In order to avoid selection for resistance while providing adequate disease control, one should choose the fungicide ratio r_{B} and the total concentration C in the following way:

1. measure the pharmacological properties of both fungicides to determine k_{k}, and C_{50}.
2. determine the degree of pharmacological interaction u between fungicides A and B.
3. measure the fitness cost of resistance ρ_{r}
4. choose the proportion of the fungicide B above the threshold: $r_{\mathrm{B}} > r_{\mathrm{Bc}}$, such that the resistance is not favored by selection at any total fungicide concentration C.
5. choose the total fungicide concentration, according to the desired level of disease control (see Fig. 4).

Box 1: How to determine an optimal mixture of fungicides.

fungicides in the mixture can be determined at which resistant strains of all pathogens are not favored by selection.

The optimal proportion of fungicides in the mixture depends on the fitness cost of resistance, the degree of resistance, dose-response parameters of the fungicides and the degree of their pharmacological interaction. All these factors affect the optimal fungicide proportion which may vary from one host-pathogen combination to another. Hence, the end user should be able to mix fungicides in a controlled way (e. g. a tank mixture) in order to control a single pathogen or a group of pathogens common in the region. Thus, we agree with [5] that a tank mixture has an advantage compared to a formulated mixture (a mixture produced by the fungicide manufacturer).

The theoretical results presented here need to be tested experimentally. We suggest to measure the amount of disease and the frequency of resistance as functions of time at different proportions of the high and low risk fungicides in the mixture. In this way, the existence of an optimal proportion of fungicides in the mixture could be tested empirically in cases where significant fitness costs are associated with fungicide resistance. The model prediction that the mixture will be ineffective if fitness costs are absent can also be tested in this way.

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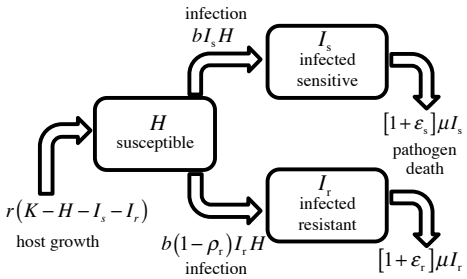

Figure 1: Scheme of the model in Eqs. (1)-(3).

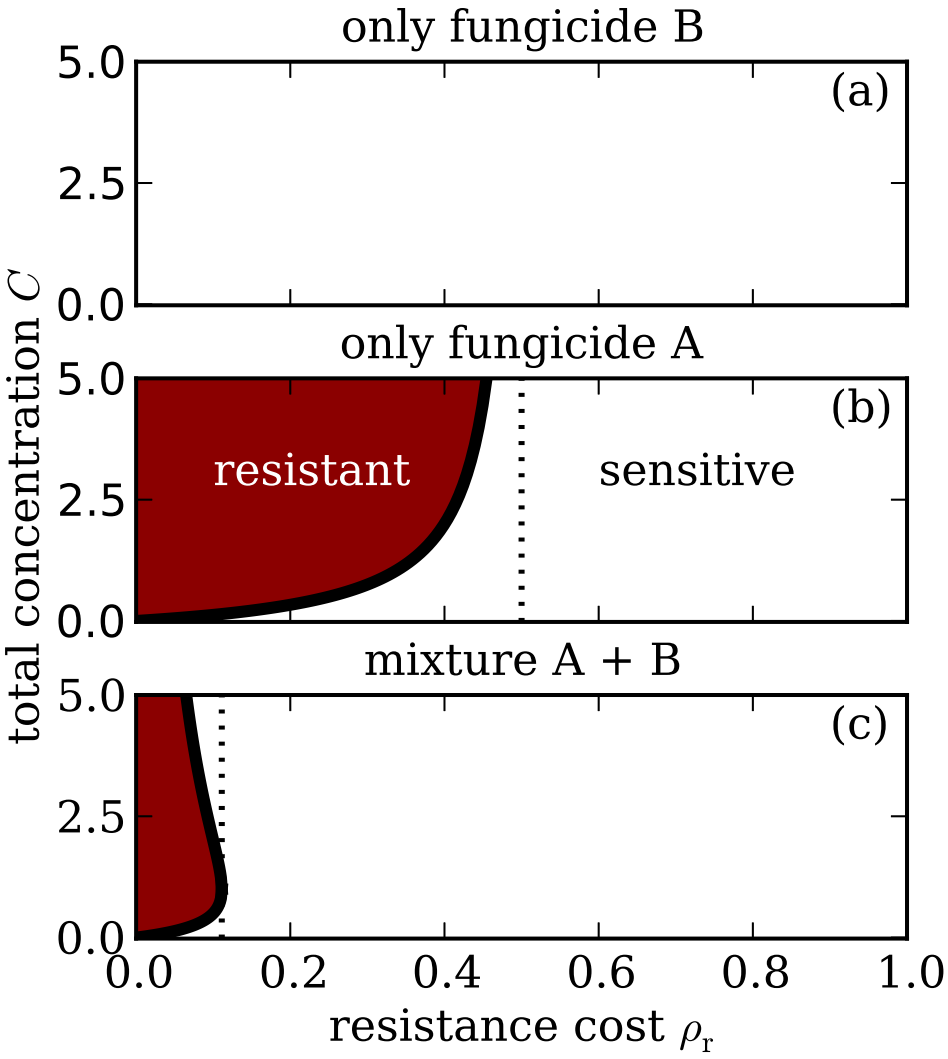

Figure 2: Outcomes of the competition between the sensitive and resistant pathogen strains depending on the fitness cost of resistance ρ_r and the fungicide concentration C when treated with a single fungicide B at ($C_B = C$, panel (a)), a single fungicide A ($C_B = C$, panel (b)) and the combination of fungicides A and B ($C_A = C_B = C/2$, panel (c)). The range of the total fungicide concentration C and the fitness cost of resistance ρ_r, in which resistant strain is favored is shown in dark red. The range where selection favors the sensitive strain is shown in white. The vertical dashed line and the solid black curve in panel (b) are plotted according to Eq. (A.21) and Eq. (A.22) in Appendix A.2, respectively. The vertical dashed line and the solid black line in panel (c) are drawn according to Eq. (A.13) and Eq. (A.14) in Appendix A.1, respectively, at $\gamma_s = 1$, $\gamma_r = 1/2$. Fungicides are assumed to have zero interaction ($u = 0$) and the resistant strain is assumed to be fully protected from fungicide A ($\alpha = 0$), the fungicide dose-response parameters are $k_k = 1$, $C_{50} = 1$.

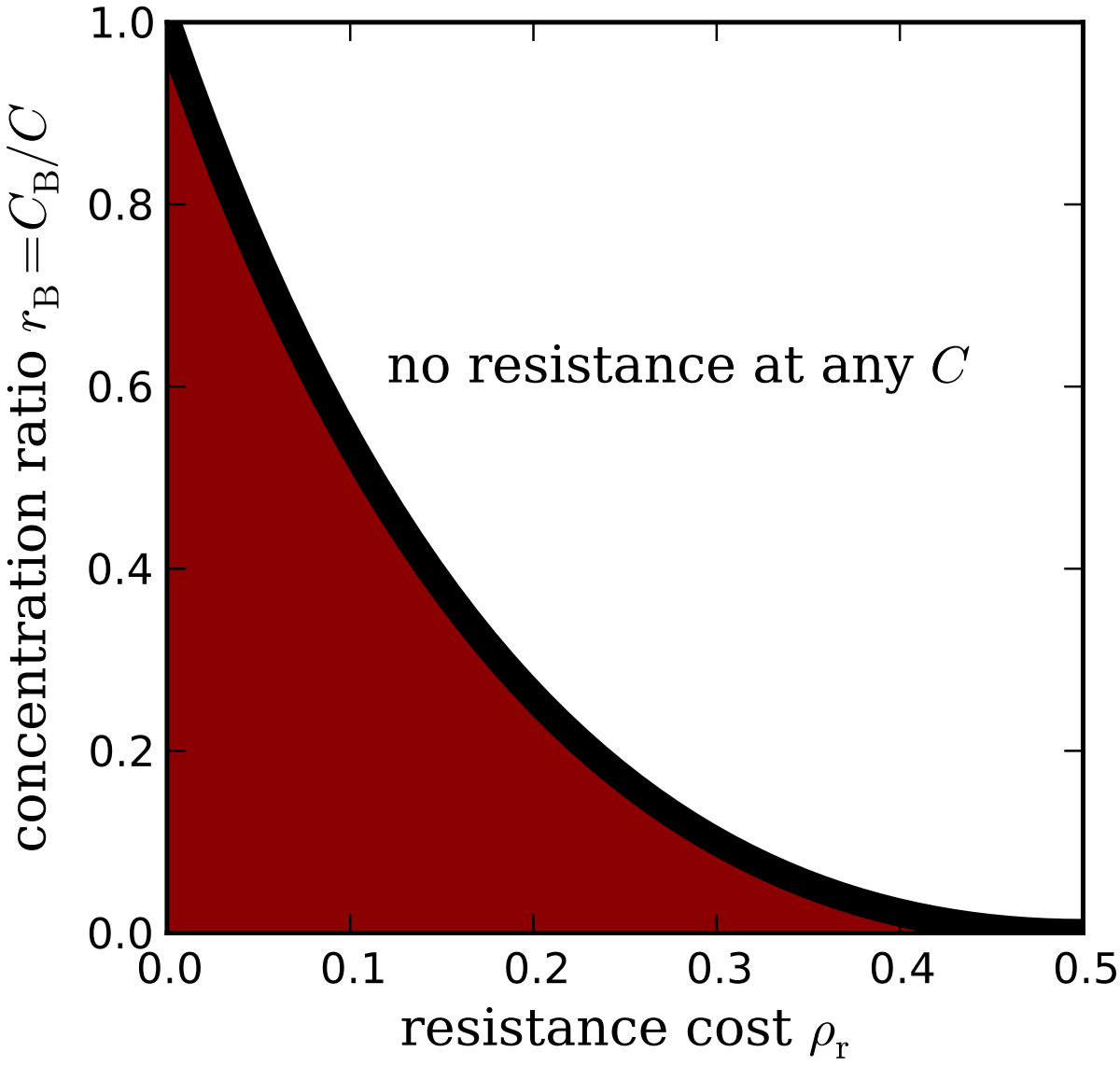

Figure 3: The critical proportion r_{Bc} of fungicide B (low risk fungicide) in the mixture, above which there is no selection for the resistant strain at any total fungicide concentration C, plotted according to Eq. (A.17) as a function of the resistance cost ρ_{r}, assuming no pharmacological interaction ($u = 0$) and full resistance ($\alpha = 0$).

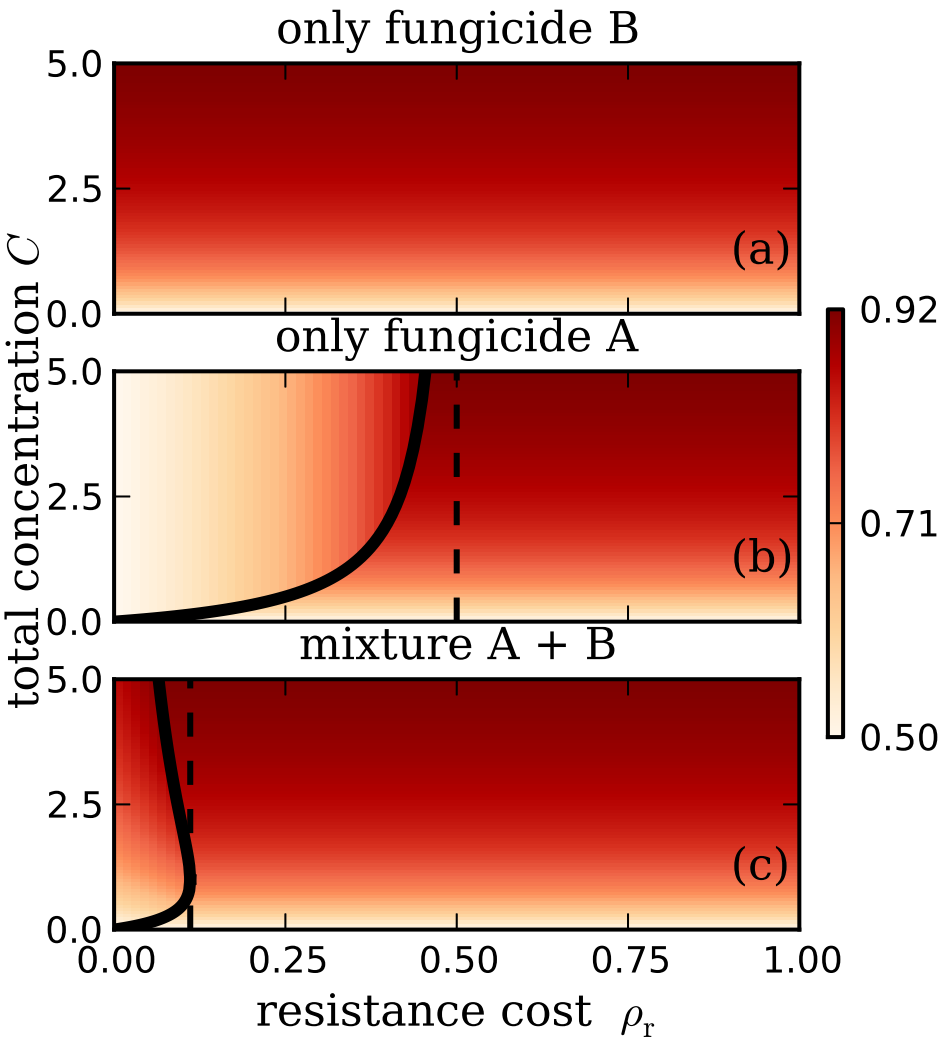

Figure 4: Treatment benefit as a function of fungicide concentration C and fitness cost of resistance ρ_r, plotted according to Eq. (A.25) in panel (a), according to Eq. (A.26) in panel (b) and according to Eq. (A.27) in panel (b). Treatment with fungicide B is shown in panel (a). Treatment with fungicide A is shown in panel (b). Treatment with a mixture of A and B at equal concentrations ($r_B = 1/2$) is shown in panel (c). The solid and dashed black in panels (b) and (c) are the same as in Fig. 2. Fungicides are assumed to have zero interaction ($u = 0$) and the resistant strain is assumed to be fully protected from fungicide A ($\alpha = 0$). The maximum killing rate $k_k = 1$, the basic reproductive number of the sensitive strain without fungicide treatment $R_{0s}(C = 0) = bK/\mu = 2$.

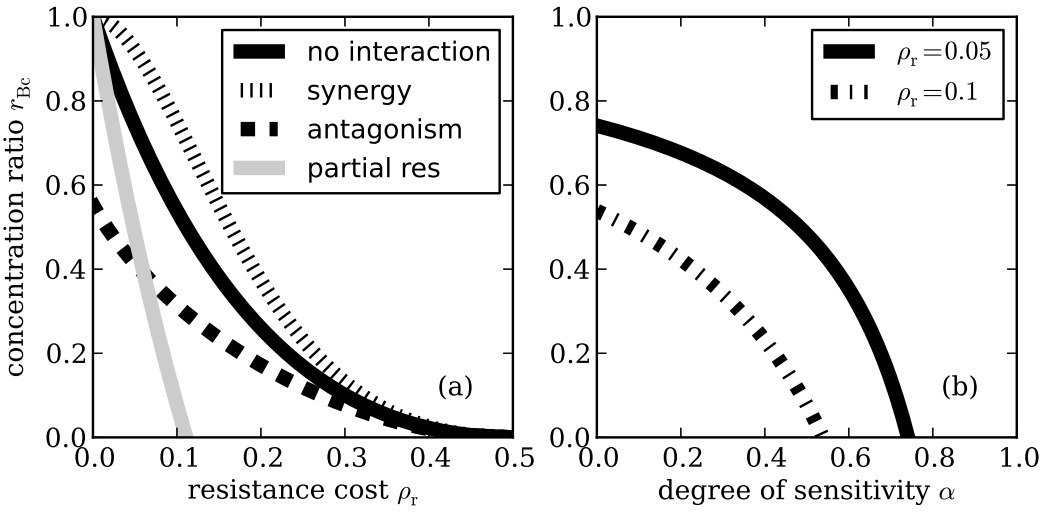

Figure 5: The effect of pharmacological interaction and partial resistance on r_{Bc}, the critical ratio of the fungicide B. r_{Bc} is plotted as a function of the fitness cost of resistance ρ_{r} (left panel), according to Eq. (A.13) for the case of no interaction between the fungicides $u = 0$ (solid, the same as the curve in Fig. 3), synergy $u = 0.9$ (dotted), and antagonism $u = -0.9$ (dashed) for the case of perfect resistance $\alpha = 1$. The case of partial resistance at no interaction ($\alpha = 0.5$, $u = 0$) is shown as a light grey curve. r_{Bc} is shown as a function of the degree of fungicide sensitivity α at $\rho_{\mathrm{r}} = 0.05$ (solid) and $\rho_{\mathrm{r}} = 0.1$ (dash-dotted) also according to Eq. (A.13) in the right panel.

A. Electronic Supplementary Material

A.1. Model equations

In order to elucidate the assumptions we made in Sec. 2, we consider a more general system of equations, which describes the change in time of the same quantities as in Eqs. (1)-(3): the amount of susceptible host tissue H, the amount of host tissue infected with the sensitive pathogen strain I_s and the amount of host tissue infected with the resistant pathogen strain I_r

$$\frac{dH}{dt} = r(K - H - I_\mathrm{s} - I_\mathrm{r}) - b\left[(1-\rho_\mathrm{s})I_\mathrm{s} + (1-\rho_\mathrm{r})I_\mathrm{r}\right]H, \tag{A.1}$$

$$\frac{dI_\mathrm{s}}{dt} = b(1-\rho_\mathrm{s})HI_\mathrm{s} - \left[1 + \varepsilon_\mathrm{s}(C_\mathrm{A}, C_\mathrm{B})\right]\mu I_\mathrm{s}, \tag{A.2}$$

$$\frac{dI_\mathrm{r}}{dt} = b(1-\rho_\mathrm{r})HI_\mathrm{r} - \left[1 + \varepsilon_\mathrm{r}(C_\mathrm{A}, C_\mathrm{B})\right]\mu I_\mathrm{r}, \tag{A.3}$$

where, the function $\varepsilon_\mathrm{s}(C_\mathrm{A}, C_\mathrm{B})$ describes the effect of the application of the mixture fungicides A and B with concentrations C_A and C_B on the death rate of the sensitive pathogen strain and the function $\varepsilon_\mathrm{r}(C_\mathrm{A}, C_\mathrm{B})$ describes the effect of this mixture on the death rate of the resistant strain:

$$\varepsilon_\mathrm{s}(C_\mathrm{A}, C_\mathrm{B}) = k_\mathrm{k}\frac{\alpha_{\mathrm{s,A}}C_\mathrm{A} + \alpha_{\mathrm{s,B}}C_\mathrm{B}}{\alpha_{\mathrm{s,A}}C_\mathrm{A} + \alpha_{\mathrm{s,B}}C_\mathrm{B} + C_{50}/\left[1 + u\sqrt{\alpha_{\mathrm{s,A}}\alpha_{\mathrm{s,B}}C_\mathrm{A}C_\mathrm{B}}/(\alpha_{\mathrm{s,A}}C_\mathrm{A} + \alpha_{\mathrm{s,B}}C_\mathrm{B})\right]}, \tag{A.4}$$

$$\varepsilon_\mathrm{r}(C_\mathrm{A}, C_\mathrm{B}) = k_\mathrm{k}\frac{\alpha_{\mathrm{r,A}}C_\mathrm{A} + \alpha_{\mathrm{r,B}}C_\mathrm{B}}{\alpha_{\mathrm{r,A}}C_\mathrm{A} + \alpha_{\mathrm{r,B}}C_\mathrm{B} + C_{50}/\left[1 + u\sqrt{\alpha_{\mathrm{r,A}}\alpha_{\mathrm{r,B}}C_\mathrm{A}C_\mathrm{B}}/(\alpha_{\mathrm{r,A}}C_\mathrm{A} + \alpha_{\mathrm{r,B}}C_\mathrm{B})\right]}. \tag{A.5}$$

The parameters $\alpha_{\mathrm{s,A}}$, $\alpha_{\mathrm{s,B}}$, $\alpha_{\mathrm{r,A}}$ and $\alpha_{\mathrm{r,B}}$ characterize the degree of sensitivity of each of the two pathogen strains (index "s" for the sensitive strain, index "r" for the resistant strain) to each of the two fungicides A and B. Their values are between zero and one. In this general case both pathogen strains are partially resistant to both fungicides.

The parameter C_{50} in Eqs. (A.4), (A.5) is modified due to pharmacological interaction between fungicides characterized by the degree of interaction u. At $u = 0$ fungicides do not interact, $u > 0$ represents synergy and $u < 0$ corresponds to antagonism. [We restrict our consideration to $u > -1$, since otherwise the term in the square brackets of Eqs. (A.4), (A.5) may become negative, which makes no sense.] This way to define pharmacological interaction between compounds is called "Loewe additivity" or "concentration addition" in the literature [46, 47]. In this approach an interaction of a compound with itself is set by definition to be additive (zero interaction). For example, when the fungicide A is mixed with itself, the resulting sham mixture is neither synergistic, nor antagonistic but has zero interaction. An alternative way to define pharmacological interaction assumes that the two compounds have independent modes of action and is

called "Bliss independence" [48] or Abbott's formula [49]. However, in this definition a compound can have a pharmacological interaction with itself, i. e. be synergistic or antagonistic.

There are several ways to introduce a deviation from the zero interaction regime, in which usually an interaction term is added to the isobologram equation [46]. We have chosen a specific form of the interaction term, which is proportional to the square root of the product of the concentrations of the two compounds [Eq. (28) in [46]]. This form allows for a simple analytical expression of the effect of the combination in Eqs. (A.4), (A.5).

We assume that the cost of resistance decreases the transmission rate b by a fixed amount $\rho_{\rm s}$ for the sensitive strain and by $\rho_{\rm r}$ for the resistant strain in Eqs. (A.1)-(A.3). We restrict our consideration here to the case when the "sensitive" pathogen strain is fully sensitive to both fungicides ($\alpha_{\rm s,A} = \alpha_{\rm s,B} = 1$) and the "resistant" strain can have varying degrees of resistance to the fungicide A ($\alpha_{\rm r,A} \equiv \alpha$, $0 \leq \alpha \leq 1$), but is fully sensitive to the fungicide B ($\alpha_{\rm r,B} = 1$). Therefore, the cost of resistance for the sensitive strain is zero $\rho_{\rm s} = 0$. Then, the fungicide dose-response functions become simpler [Eqs. (5), (6)].

In order to determine the range of fitness costs $\rho_{\rm r}$ and fungicide concentrations C, over which the sensitive strain is favored by selection we perform the linear stability analysis of the fixed points of the system Eqs. (A.1)-(A.3), which is equivalent to solving the inequalities $R_{0\rm s} > 1$, $R_{0\rm s} > R_{0\rm r}$, where

$$R_{0\rm s} = \frac{bK}{[1 + \varepsilon_{\rm s}(C_{\rm A}, C_{\rm B})]\,\mu} \tag{A.6}$$

is the basic reproductive number of the sensitive strain and

$$R_{0\rm r} = \frac{b(1-\rho_{\rm r})K}{[1 + \varepsilon_{\rm r}(C_{\rm A}, C_{\rm B})]\,\mu} \tag{A.7}$$

is the basic reproductive number of the resistant strain.

We consider then the inequality $R_{0\rm s} > R_{0\rm r}$, which is equivalent to

$$\frac{C}{C + C_{50}/\gamma_{\rm s}} < \left(\rho_{\rm r}/k_{\rm k} + \frac{C}{C + C_{50}/\gamma_{\rm r}}\right)/(1-\rho_{\rm r}), \tag{A.8}$$

where

$$\gamma_{\rm s} = 1 + u\sqrt{r_{\rm B}(1-r_{\rm B})}, \tag{A.9}$$

$$\gamma_{\rm r} = \alpha(1-r_{\rm B}) + r_{\rm B} + u\sqrt{\alpha r_{\rm B}(1-r_{\rm B})} \tag{A.10}$$

and $r_{\rm B} = C_{\rm B}/C$ is the proportion of the funcigide B in the mixture, $C = C_{\rm A} + C_{\rm B}$.

The inequality (A.8) holds at

$$\rho_{\rm r} < \rho_{\rm rb}, \;\; \text{for}\, (C < C_{\rm b1} \,\text{or}\, C > C_{\rm b2}) \tag{A.11}$$

or at

$$\rho_{\rm r} > \rho_{\rm rb}, \text{ for any value of } C. \tag{A.12}$$

Here,

$$\rho_{\rm rb} = \frac{k_{\rm k}(\gamma_{\rm s}-\gamma_{\rm r})\left(\gamma_{\rm s}+\gamma_{\rm r}+\gamma_{\rm s}k_{\rm k}-2\sqrt{\gamma_{\rm r}\gamma_{\rm s}(1+k_{\rm k})}\right)}{(\gamma_{\rm r}-\gamma_{\rm s}(1+k_{\rm k}))^2}, \tag{A.13}$$

$$C_{b1,2} = \frac{C_{50}}{2\gamma_{\rm s}\gamma_{\rm r}\rho_{\rm r}(1+k_{\rm k})}\left[\gamma_{\rm s}(\rho_{\rm r}-k_{\rm k}+k_{\rm k}\rho_{\rm r})+\gamma_{\rm r}(k_{\rm k}+\rho_{\rm r})\pm\sqrt{D}\right], \tag{A.14}$$

where

$$D = \gamma_{\rm s}^2\left(k_{\rm k}(\rho_{\rm r}-1)+\rho_{\rm r}\right)+\gamma_{\rm r}^2(k_{\rm k}+\rho_{\rm r})^2+2\gamma_{\rm s}\gamma_{\rm r}\left(k_{\rm k}^2(\rho_{\rm r}-1)-\rho_{\rm r}^2-k_{\rm k}\rho_{\rm r}^2\right). \tag{A.15}$$

According to the inequality (A.12), if the fitness cost of resistance is larger than a threshold value given by Eq. (A.13), the sensitive strain has a selective advantage and the resistant strain is eliminated from the population at any fungicide concentration $C \geq 0$.

For the case of no interaction between fungicides ($u = 0$) and perfect resistance ($\alpha = 0$) the Eq. (A.13) is simplified:

$$\rho_{\rm rb} = k_k\frac{(1-r_B)(1+k_k+r_B-2\sqrt{r_B(1+k_k)})}{(1+k_k-r_B)^2}. \tag{A.16}$$

We then solve the inequality $\rho > \rho_{\rm rb}$ with respect to $r_{\rm B}$ and find that it is fulfilled at $r_{\rm B} > r_{\rm Bc}$, where

$$r_{\rm Bc} = \frac{\rho_{\rm r}^2+k_k^2(1-\rho_{\rm r})+k_k\rho_{\rm r}(\rho_{\rm r}-2\sqrt{(1+k_k)(1-\rho_{\rm r})})}{(k_k+\rho_{\rm r})^2} \tag{A.17}$$

It represents the critical proportion of the fungicide B in the mixture above which the resistant strain is not favored by selection (Fig. 3).

A.2. Selection for resistance at no interaction between fungicides

When only the high risk fungicide (fungicide A) is applied with the concentration $C_{\rm A}$, we set $r_{\rm B} = 0$ in Eq. (A.9) and Eq. (A.10) to obtain $\gamma_{\rm s} = 1$, $\gamma_{\rm r} = \alpha$. Then, the following expressions are obtained for the threshold value of the resistance cost from Eq. (A.13)

$$\rho_{\rm rb} = \frac{k_{\rm k}(1-\alpha)\left(1+\alpha+k_{\rm k}-2\sqrt{\alpha(1+k_{\rm k})}\right)}{(\alpha-k_{\rm k}-1)^2}, \tag{A.18}$$

and the fungicide concentration from Eq. (A.14)

$$C_{b1,2} = \frac{C_{50}}{2\alpha\rho_{\rm r}(1+k_{\rm k})}\left[(\rho_{\rm r} - k_{\rm k}(1-\rho_{\rm r})) + \alpha(k_{\rm k}+\rho_{\rm r}) \pm \sqrt{D}\right], \tag{A.19}$$

where

$$D = \alpha^2(k_{\rm k}+\rho_{\rm r})^2 - \alpha\left(k_{\rm k}^2(1-\rho_{\rm r}1) + \rho_{\rm r}^2 + k_{\rm k}\rho_{\rm r}^2\right) + \rho_{\rm r} - k_{\rm k}(1-\rho_{\rm r}). \tag{A.20}$$

In the simpler case of full resistance we take the limit $\alpha \to 0$. Then, by taking this limit in Eq. (A.18), Eq. (A.19) and Eq. (A.20) we obtain for the threshold values of the fitness cost and the fungicide concentration

$$\rho_{\rm rb} = k_{\rm k}/(k_{\rm k}+1), \tag{A.21}$$

$$C_{\rm b} = C_{50}\frac{\rho_{\rm r}}{k_{\rm k}(1-\rho_{\rm r}) - \rho_{\rm r}}. \tag{A.22}$$

In this case the sensitive strain dominates at $C < C_{\rm b}$ if $\rho_{\rm r} < \rho_{\rm rb}$ or at any positive values of C if $\rho_{\rm r} > \rho_{\rm rb}$ [white area in Fig. 2(b)].

When only the low risk fungicide (fungicide B) is applied, we set $r_{\rm B} = 1$ in the inequality (A.8) and obtain

$$\frac{C}{C+C_{50}} < \rho_{\rm r}/k_{\rm k} + \frac{C}{C+C_{50}}. \tag{A.23}$$

This inequality holds and the sensitive strain dominates for all positive values of $\rho_{\rm r}$ and C at which $R_{0\rm s} > 1$

Consider the case when the two fungicides A and B are applied together at an arbitrary mixing ratio $r_{\rm B}$, assuming no pharmacological interaction ($u = 0$) and perfect resistance of the resistant strain to the fungicide A ($\alpha = 0$). In this case, $\gamma_{\rm s} = 1$ and $\gamma_{\rm r} = r_{\rm B}$. Substituting these values in Eq. (A.13), Eq. (A.14) and Eq. (A.15) gives the same expressions as in Eq. (A.18), Eq. (A.19) and Eq. (A.20), but with α substituted by $r_{\rm B}$.

A.3. Expressions for the treatment benefit

The treatment benefit is defined as the ratio between the amount of susceptible hosts $H(t)$ when both the disease and treatment are present and the amount of healthy hosts at no disease $B(t) = H(t)/H_{\rm nd}(t)$ (see Sec. 3.3). We consider here its equilibrium value over long time scales given by

$$B(t \to \infty) = B^* = \frac{H^*}{K}, \tag{A.24}$$

where H^* is the equilibrium amount of healthy hosts and K is the host carrying capacity, and assume full resistance ($\alpha = 0$).

When only the fungicide B is applied at a concentration C [Fig. 4(a)], the basic reproductive number of the sensitive pathogen strain always exceeds the one for the resistant strain $R_{0\mathrm{s}} > R_{0\mathrm{r}}$. Therefore, the resistant mutants are eliminated in the long run and the amount of the healthy host tissue is equal to $H^* = \mu\,[1 + \varepsilon(C)]$, where $\varepsilon(C)$ is given by Eq. (4). Then, according to Eq. (A.24), the treatment benefit is

$$B^*(C) = \mu\,[1 + \varepsilon(C)]\,/(bK). \tag{A.25}$$

It grows with the fungicide concentration and saturates, since the function $\varepsilon(C)$ saturates.

Application of the fungicide A alone at a concentration C may favor either resistant or sensitive pathogen strain depending on the fitness cost of resistance ρ_{r} and the fungicide concentration C [see Fig. 2(b)]. The treatment benefit in this case is

$$B^*(C,\rho_{\mathrm{r}}) = \begin{cases} \mu\,[1 + \varepsilon(C)]\,/(bK), & \text{for } (\rho_{\mathrm{r}} < k_k/(1+k_k) \text{ and } C < C_{\mathrm{b}}) \\ & \text{or } (\rho_{\mathrm{r}} > k_k/(1+k_k) \text{ and } \forall C), \\ \mu/\,[b(1-\rho_{\mathrm{r}})K]\,, & \text{for } \rho_{\mathrm{r}} < k_k/(1+k_k) \text{ and } C > C_{\mathrm{b}}, \end{cases} \tag{A.26}$$

where the C_{b} is given by Eq. (A.22).

Now, consider application of both fungicides in a mixture at equal concentrations ($r_{\mathrm{B}} = 1/2$), assuming no interaction between fungicides ($u = 0$). In this case, again either resistant or sensitive pathogen strain will dominate the population depending on the fitness cost ρ_{r} and the total fungicide concentration C [see Fig. 2(c)]. The treatment benefit now has the following expression

$$B^*(C,\rho_{\mathrm{r}}) = \begin{cases} \mu\,[1 + \varepsilon(C)]\,/(bK), & \text{for } (\rho_{\mathrm{r}} < \rho_{\mathrm{rb}} \text{ and } (C < C_{\mathrm{b1}} \text{ or } C > C_{\mathrm{b2}})) \\ & \text{or } (\rho_{\mathrm{r}} > \rho_{\mathrm{rb}} \text{ and } \forall C), \\ \mu\,[1 + \varepsilon(C/2)]\,/\,[b(1-\rho_r)K]\,, & \text{for } \rho_{\mathrm{r}} < \rho_{\mathrm{rb}} \text{ and } C_{\mathrm{b1}} < C < C_{\mathrm{b2}}, \end{cases} \tag{A.27}$$

where ρ_{rb}, C_{b1} and C_{b2} and are given by Eq. (A.13) and Eq. (A.14) at $\gamma_{\mathrm{s}} = 1$, $\gamma_{\mathrm{r}} = 1/2$. The boundary value of the resistance cost has then a simpler expression $\rho_{\mathrm{rb}} = k_{\mathrm{k}}(2k_k - 2\sqrt{2(1+k_k)} + 3)/(1+2k_k)^2$. The treatment benefit $B^*(C,\rho_{\mathrm{r}})$ is shown as a function of the fungicide concentration C and the fitness cost of resistance ρ_{r} in Fig. 4 for the three cases discussed above, according to Eqs. (A.25)-(A.27).

A.4. Selection for resistance is delayed by applying a fungicide mixture

If the fungicide resistance is not associated with a fitness cost, then the resistant strain is favored by selection and eventually dominates the population whenever the high risk fungicide is applied alone or in a mixture with the low risk fungicide [Fig. 2(b,c)]. However, for a given value of the total fungicide concentration C, the selection for resistance

slows down when applying the fungicide mixture as compared to the treatment with the high risk fungicide alone [as seen from time-dependent numerical solutions of the model Eqs. (1)-(3)] in agreement with the findings of [5].

In order to understand this result we consider the dynamics of the frequency of the resistant pathogen strain $p(t) = I_{\rm r}/(I_{\rm r} + I_{\rm s})$. The rate of its change is obtained from Eqs. (1)-(3) [9]

$$\frac{dp}{dt} = sp(1-p), \tag{A.28}$$

where

$$s = \mu\left[\varepsilon_{\rm s}(C, r_{\rm B}) - \varepsilon_{\rm r}(C, r_{\rm B})\right] - \rho_{\rm r} bH(t) \tag{A.29}$$

is the selection coefficient [a similar expression was found in [50]]. Here $\varepsilon_{\rm s}(C, r_{\rm B})$ and $\varepsilon_{\rm r}(C, r_{\rm B})$ are given by Eqs. (5), (6) and $r_{\rm B}$ is the proportion of the fungicide B in the mixture.

Assuming a zero fitness cost ($\rho_{\rm r} = 0$), no pharmacological interaction ($u = 0$) and full resistance ($\alpha = 0$), the selection coefficient can be written as

$$s = \mu\left[\varepsilon(C) - \varepsilon(r_{\rm B}C)\right]. \tag{A.30}$$

In this case, the selection coefficient does not depend on time and the Eq. (A.28) has a simple analytical solution:

$$p(t) = \frac{p_0 \exp(st)}{1 + p_0\left[\exp(st) - 1\right]}, \tag{A.31}$$

where $p_0 = p(t = 0)$. At $s > 0$, the function $p(t)$ grows monotonously and tends to one at large times. The rate, at which it grows is determined by the magnitude of the selection coefficient s.

One can see from Eq. (A.30) that when the high risk fungicide is applied alone ($r_{\rm B} = 0$), the selection coefficient is larger than when it is mixed with a low risk fungicide ($0 < r_{\rm B} < 1$) at the same total fungicide concentration C. Hence, $s(r_{\rm B} = 0, C) > s(r_{\rm B} > 0, C)$. This is because the function $\varepsilon(r_{\rm B}C)$ has positive values for any $r_{\rm B} > 0$. Thus, the selection for the resistant strain (against the sensitive strain) is delayed when a mixture of high risk and low risk fungicides is applied compared to treatment with the high risk fungicide alone.

A.5. Generalization of the model

So far we assumed that resistance cost manifests in the transmission rate b of the resistant pathogen strain and the fungicide increases the pathogen death rate μ. We performed the same analysis for the three remaining cases possible in the model: When (i) both resistance cost and the fungicide affect the pathogen death rate according to $\mu \to \mu(1 + \rho_{\rm r} + \varepsilon_{\rm r}(C, r_{\rm B}))$ for the resistant strain and $\mu \to \mu(1 + \varepsilon_{\rm s}(C, r_{\rm B}))$ for the sensitive strain; (ii) both resistance cost and the fungicide affect the infection rate $b \to b(1 - \rho_{\rm r} - \varepsilon_{\rm r}(C, r_{\rm B}))$ for the resistant strain and $b \to b(1 - \varepsilon_{\rm s}(C, r_{\rm B}))$ for the sensitive strain; (iii) resistance cost

affects the death rate of the resistant pathogen strain $\mu \to \mu(1+\rho_{\rm r})$, while the fungicide affects the infection rate of both resistant and sensitive strains $b \to b(1-\varepsilon_{\rm r,s}(C, r_{\rm B}))$. We have found that although the mathematical expressions for the results have a different form in these cases, all the conclusions remain the same, so that the results do not depend on whether the fungicide and the resistance cost manifest in the infection rate b or in the pathogen death rate μ. Moreover, we have done the same analysis using a fungicide dose-response function different from Eq. (4), namely using an exponential function and again found that all the conclusions remain the same.

This generalization applies to the results at equilibrium, but the time-dependent solutions of Eqs. (1)-(3) may behave differently depending on how the fungicide and the fitness cost affect the pathogen life cycle and the form of the fungicide dose-response function. This is an interesting topic for further investigations, but lies beyond the scope of this study.

A.6. Fungicide mixture versus alternation

It was previously discussed [45] that in the presence of a fitness cost the alternation of fungicides can be effective, but we have shown here that fungicide mixtures can also be effective in this case. When using an alternation strategy, the period of selection during which the resistant strain is favored in the presence of the high risk fungicide is followed by a period during which selection favors the sensitive strain in the absence of this fungicide. The latter period is typically much longer because the selection pressure induced by the high risk fungicide is much larger than that induced by the fitness cost of resistance. Hence, one needs to wait for quite a long time before the resistant strain disappears and the high risk fungicide can be used again. Moreover, there are times during which the frequency of the resistant strain becomes large (at the end of the period of the application of the high risk fungicide), which increases the risk that resistance will spread to other regions. Both of these disadvantages are avoided by using a mixture where the proportion of the low risk fungicide is above a critical value determined here (Fig. 3). In this case there is no need to delay the application of the high risk fungicide and the frequency of the resistant strain does not rise above the mutation- or migration-selection equilibrium because the mixture does not induce selection for resistance.

A.7. The risk of double resistance

Although we do not consider the possibility of double resistance in our model, by applying an optimal proportion of fungicides in the mixture as suggested here, one would prevent selection for resistance to the high risk fungicide. Consequently, the risk of development of double resistance would be reduced. For both sexually and asexually reproducing pathogens, there are three pathways for generating double resistance: (i) A-resistant mutants are produced first and then a proportion of them acquires also B-resistance by spontaneous mutation (ii) B-resistant mutants are generated first and subsequently acquire A-resistance and (iii) double resistance is generated directly from the wild-type. In this case, by preventing selection for A-resistance, one removes only

the pathway (i) to double resistance. If a pathogen is able to reproduce sexually, then a much more likely scenario for the double resistance to emerge is through recombination. For the recombination to occur, both singly resistant strains (A-resistant and B-resistant) would need to be present in the population at significant frequencies. Hence, preventing selection for A-resistance would diminish the probability of the emergence of double resistance by recombination. Thus, our findings would also help to significantly reduce the risk of development of double resistance, especially in sexually reproducing pathogens.